REMARKS

Claims 1-22 are pending. No new matter has been added by way of the present amendment. For instance, each of the independent claims have been amended to remove the "identifying" step. This does not add new matter.

In view of the following remarks Applicant respectfully requests that the Examiner withdraw all rejections and allow the currently pending claims.

Issues Under 35 U.S.C. §102(b)

The Examiner has rejected claims 1-5, 7, 10, 11, 13, 16, 17, 19 and 22 under 35 U.S.C. §102(b) as being anticipated by Fuhrman, *The American Journal of Clinical Nutrition*, Vol. 66, Pages 267-275 (1997)(hereinafter referred to as Fuhrman). Applicant respectfully traverses this rejection.

Applicant respectfully submits that Fuhrman fails to suggest or disclose the currently claimed subject matter. The rejected claims are drawn to methods for lowering a risk factor in a patient, lowering at least two risk factors simultaneously or preventing a patient from suffering from a particular condition. These methods involve administering an effective amount of a licorice extract which is water-insoluble and free from

glycyrrhizinic acid to the patient. However, Fuhrman fails to suggest or disclose this specific subject matter.

The Examiner has stated that "[l]owering the risk factors in a patient are inherent in the disclosure of Fuhrman because by reducing the potential of oxidative damage by free radicals a patient is at a lower risk of getting a disease such as blood pressure or blood glucose concentration." Applicant respectfully disagrees with the Examiner's rationale. That is, Applicant asserts that the Examiner's rationale is based upon an unproven hypothesis and insufficient for the establishment of a theory of either inherency or obviousness. Applicant has attached evidence which rebuts the theory of inherency suggested by the Examiner. For instance, the Examiner is referred to the following two articles attached hereto:

1) Attached is a copy of an article, published in *Current Controlled Trials in Cardiovascular Medicine* 3(1), p5 (2002), titled: "Long-term vitamin E supplementation fails to reduce lipid peroxidation in people at cardiovascular risk: analysis of underlying factors" (see attached copy). Even the title itself is enough to demonstrate that lowering a risk factor is not inherent to consumption of an anti-oxidant.

2) Also attached is a copy of an article titled "Can garlic reduce levels of serum lipids? A controlled clinical study" published in *The American Journal of Medicine*, Vol. 94. This article finds that while garlic (which is a well known antioxidant) lowers total blood cholesterol and serum LDL levels, "there were no significant changes in HDL, triglycerides, serum glucose, blood pressure, and other monitored parameters".

Based upon this distinction alone, the Examiner has failed to present a valid case of anticipation.

A theory of inherency must be supported by facts and/or technical reasoning that reasonably support a determination that the allegedly inherent characteristic necessarily flows from the teachings of the prior art. *Ex parte Levy* 17 USPQ2d 1461 (BPAI 1990) (emphasis added). In order for prior art to anticipate a claimed compound on the ground it is inherently produced in a prior art process, the inherency must be certain. *Glaxo, Inc. v. Novopharm Ltd.*, (EDNC 1993) 830 F. Supp 871, 29 USPQ2d 1126; *Ex parte Cyba* (POBA 1966) 155 USPQ 756; *Ex parte McQueen* (POBA 1958) 123 USPQ 37. The fact that a prior art article may inherently have the characteristics of the claimed product is not sufficient. *Ex parte Skinner* (BPAI 1986) 2 USPQ2d 1788. Inherency must be a necessary result and not merely a possible

result. *In re Oelrich* (CCPA 1981) 666 F2d 578, 212 USPQ 323; *Ex parte Keith et al.* (POBA 1966) 154 USPQ 320.

Accordingly, in the present instance, the evidence does not support an assertion that the cited art inherently achieves the current claim limitations. If the Examiner wishes to maintain this assertion, Applicant requests that the Examiner provide documentary evidence. In this regard the Examiner is requested to refer to 37 C.F.R. §1.104(c)(2) and In re Zurko, 59 U.S.P.Q. 2d 1693, 1697 (Fed. Cir. 2001). Alternatively, if the Examiner is relying upon personal knowledge to support the finding of what is known in the art, the Examiner is respectfully requested to provide an affidavit or declaration setting forth specific factual statements and explanation to support such a finding. In this regard the Examiner is referred to 37 C.F.R. §1.104(d)(2).

In summary, Applicant respectfully submits that the Examiner has failed to present a valid *prima facie* case of anticipation. Reconsideration and withdrawal of this rejection is respectfully requested.

Issues Under 35 U.S.C. §103(a)

The Examiner has rejected claims 6, 8, 12, 14, 18 and 20 under 35 U.S.C. §103(a) as being obvious over Fuhrman. Applicant respectfully traverses.

The Examiner asserts that is would have been obvious to one of ordinary skill in the art at the time of the claimed invention being made to select the licorice extract of Fuhrman and provide for treatment methods of inflammation and LDL levels. The Examiner again relies on the theory that the licorice extract eliminates free radical oxidation which causes inflammation and cardiovascular disease to which high blood pressure is related. Applicant again takes issue with the Examiner's hypothesis concerning reducing the potential of oxidated damage by free radicals in a patient. As indicated above, this is not a proven theory upon which the Examiner can rely for anticipation, or in the present instance, for a theory of obviousness based upon inherency. Based upon these distinctions above, the Examiner has failed to present a valid *prima facie* case of obviousness.

Even absent the above distinction, Applicant submits that Fuhrman teaches away from the present invention. For instance, Fuhrman reports that the consumption of licorice extract by humans has not been found to have any significant influence plasma cholesterol, LDL concentration, or any other medical characteristics examined (see the paragraph bridging the left and right column of page 268 of Fuhrman). Thus, this teaches away from the claimed invention.

Further, regarding anti-inflammatory influence of a

licorice, Fuhrman discloses (at page 268, lines 14-18) that it is attributed in the art to glycyrrhizinic acid, of which the extract recited in the present claims is free. Thus, Fuhrman again teaches away from the presently claimed invention.

At most the Examiner's rejection amounts to an "obvious to try" standard, which is improper in the presentation of a *prima facie* case of obviousness. "Obvious to try" is not a valid test of patentability. In re Mercier, 185 USPQ 774 (CCPA 1975); see also Hybritech Inc. v. Monoclonal Antibodies, 231 USPQ 81 (Fed. Cir. 1986). Accordingly, Applicant respectfully submits that the Examiner has failed to present a valid *prima facie* case of obviousness. Reconsideration and withdrawal of this rejection is respectfully requested.

The Examiner has rejected claims 9, 15 and 21 under 35 U.S.C. §103(a) as being obvious over Fuhrman in view of "admitted prior art (see specification at page 1, lines 10-12)." Applicant respectfully traverses this rejection.

As indicated above, the prior art of Fuhrman, even in view of what the Examiner has designated as "admitted" prior art, fails to suggest or disclose a method for treating a patient suffering from a condition listed in claim 9, such as a hypertension. The Examiner has failed to present evidence directed to the administration of an effective amount of a

licorice extract which is water-insoluble and free from glycyrrhizinic acid. Accordingly, Applicant respectfully submits that there exists no *prima facie* case of obviousness. Reconsideration and withdrawal this rejection is respectfully requested.

In view of the above, Applicant respectfully submits that the Examiner has failed to present either a case of anticipation or *prima facie* case of obviousness. Accordingly, the Examiner is respectfully requested to withdraw all rejections and allow the currently pending claims.

If the Examiner has any questions or comments, please contact Craig A. McRobbie, Registration No. 42,874 at the offices of Birch, Stewart, Kolasch & Birch, LLP.

If necessary, the Commissioner is hereby authorized in this, concurrent, and future replies, to charge payment or credit any overpayment to Deposit Account No. 02-2448 for any additional

fees required under 37 C.F.R. § 1.16 or under § 1.17; particularly, extension of time fees.

Respectfully submitted,

BIRCH, STEWART, KOLASCH & BIRCH, LLP

By _____ #42,874
Marc S. Weiner
Reg. No. 32,181

MSW/CAM:cms

P. O. Box 747
Falls Church, VA 22040-0747
(703) 205-8000

Attachments: *Current Controlled Trials in Cardiovascular Medicine*
The American Journal of Medicine

Current Controlled Trials in Cardiovascular Medicine

BioMed Central

Research article

Long-term vitamin E supplementation fails to reduce lipid peroxidation in people at cardiovascular risk: analysis of underlying factors

Chiara Chiabrando*[1], Fausto Avanzini[2], Claudia Rivalta[1], Fabio Colombo[2], Roberto Fanelli[1], Gaetana Palumbo[3] and Maria Carla Roncaglioni[2] for PPP Collaborative Group on the antioxidant effect of vitamin E

Address: [1]Department of Environmental Health Sciences, Istituto di Ricerche Farmacologiche 'Mario Negri', via Eritrea 62, 20157 Milano, Italy, [2]Department of Cardiovascular Research, Istituto di Ricerche Farmacologiche 'Mario Negri', via Eritrea 62, 20157 Milano, Italy and [3]Divisione di Medicina V piano, Azienda Ospedaliera Ospedale San Carlo Borromeo, Via Pio II 3, 20153 Milano, Italy

E-mail: Chiara Chiabrando* - chiabrando@marionegri.it; Fausto Avanzini - avanzini@marionegri.it; Claudia Rivalta - rivalta@marionegri.it; Fabio Colombo - fabio@marionegri.it; Roberto Fanelli - fanelli@marionegri.it; Gaetana Palumbo - geetana.palumbo@tiscalinet.it; Maria Roncaglioni - roncaglioni@marionegri.it

*Corresponding author

Published: 19 March 2002

Current Controlled Trials in Cardiovascular Medicine 2002, 3:5

Received: 15 January 2002
Accepted: 19 March 2002

This article is available from: http://cvm.controlled-trials.com/content/3/1/5

Keywords: Vitamin E, cardiovascular prevention, lipid peroxidation, F2-isoprostane, hypertension

Abstract

Background: Antioxidant supplementation with vitamin E had no effect in the prevention of cardiovascular diseases (CVD) in three recent large, randomized clinical trials. In order to reassess critically the role of vitamin E in CVD prevention, it is important to establish whether these results are related to a lack of antioxidant action.

Methods: We examined the *in vivo* antioxidant effect of vitamin E (300 mg/day for about three years) in 144 participants in the Primary Prevention Project (females and males, aged ≥ 50 y, with at least one major CV risk factor, but no history of CVD). Urinary 8-epi-$PGF_{2\alpha}$ (isoprostane $F_{2\alpha}$-III or 15-F_{2t}-isoP), a validated biomarker of lipid peroxidation, was measured by mass spectrometry.

Results: Urinary excretion of 8-epi-$PGF_{2\alpha}$ [pg/mg creatinine, median (range)] was 141 (67–498) in treated and 148 (76–561) in untreated subjects ($p = 0.10$). Taking into account possible confounding variables, multiple regression analysis confirmed that vitamin E had no significant effect on this biomarker. Levels of 8-epi-$PGF_{2\alpha}$ were in the normal range for most subjects, except smokers and those with uncontrolled blood pressure or hyperglycemia.

Conclusions: Prolonged vitamin E supplementation did not reduce lipid peroxidation in subjects with major cardiovascular risk factors. The observation that the rate of lipid peroxidation was near normal in a large proportion of subjects may help explain why vitamin E was not effective as an antioxidant in the PPP study and was ineffective for CVD prevention in large scale trials.

Background

The "oxidative hypothesis" of atherosclerosis proposes that oxidative modification of lipids in low-density lipoproteins (LDL) contributes to atherogenesis [1,2]. Antioxidants that are effective against lipid peroxidation should therefore reduce atherosclerosis and hence afford protection from cardiovascular diseases (CVD)[1]. In contrast to a) epidemiological evidence that antioxidants taken with the diet or as supplements reduce cardiovascular (CV) risk [3], and b) experimental data supporting its anti-atherogenic properties [4], vitamin E failed to show any beneficial effect in recent large intervention studies [5]. In two large-scale trials, long-term supplementation with vitamin E (300–400 IU/day) failed to reduce cardiovascular events in post-myocardial infarction patients (GISSI-Prevenzione [6]) and in subjects at high CV risk (HOPE [7]). In the trial conducted by our group (Primary Prevention Project, PPP [8]), vitamin E (300 mg/day) taken over three years also showed no effect on the incidence of cardiovascular events in individuals with one or more major risk factors (see Methods). Therefore, the question whether vitamin E had an effective *in vivo* antioxidant action in the populations under study in these trials is under debate [9–12].

When these studies were designed, the antioxidant efficacy of vitamin E in humans had not been demonstrated *in vivo* because of the lack of reliable methods [13]. Measurement of urinary or circulating F2-isoprostanes (iPF_2 or F_2-isoP) is now accepted as a reliable tool for evaluating the rate of lipid peroxidation *in vivo*[9,14–16]. Using urinary excretion of 8-epi-$PGF_{2\alpha}$ (also termed $iPF_{2\alpha}$-III or 15-F_{2t}-isoP) as a biomarker, it has been shown that short-term administration of vitamin E (600 mg/day for 14 days) reduced *in vivo* lipid peroxidation in some clinical settings where oxidative stress is abnormally high, e.g., diabetes mellitus (-37%), hypercholesterolemia (-58%) and cystic fibrosis (-42%) [17–19]. In contrast, vitamin E had no antioxidant activity in conditions where lipid peroxidation was normal [20]. No data are available on the antioxidant effect of longer-term supplementation with vitamin E in subjects at moderate/high cardiovascular risk.

Using a highly selective and validated mass spectrometric assay for 8-epi-$PGF_{2\alpha}$[21], we measured *in vivo* lipid peroxidation in PPP trial participants who had taken vitamin E daily for about three years *vs* those who did not take vitamin E.

Methods

PPP is a large, randomized, controlled 2 × 2 factorial trial on primary prevention of CVD [8]. It was designed to test the efficacy of long-term administration of vitamin E (synthetic α-tocopherol, 300 mg/day) and/or aspirin (100 mg/day) in preventing cardiovascular events in subjects of both sexes aged ≥ 50 years, with at least one of the following cardiovascular risk factors: old age (≥ 65 yr); hypertension (systolic blood pressure ≥ 160 mm Hg or diastolic blood pressure ≥ 95 mm Hg on at least three separate occasions); hypercholesterolemia (≥ 250 mg/dL on at least two separate occasions); diabetes mellitus (≥ 140 mg/dL fasting venous plasma glucose, on at least two separate occasions); chronic drug treatment for any of the three latter conditions; obesity (body mass index ≥ 30 kg/m^2); and premature myocardial infarction before 55 years of age in at least one parent or sibling. Patients with a history of cardiovascular events or diseases were not included. Table 1 shows the frequency of these CV risk factors in our sample.

Table 1: Major cardiovascular risk factors in the 144 subjects.

RISK FACTOR*	n(%)
Old age ≥ 65 y	38 (26)
Male sex	63 (44)
Smoking	24 (17)
Hypertension	136 (94)
Diabetes	12(8)
Hypercholesterolemia	43 (30)
Obesity	32 (22)
Family history of premature myocardial infraction	17 (12)

*See Methods for definition

Overnight urine was obtained from subjects consecutively presenting at five participating centers for a scheduled follow-up visit after at least one year of randomized treatment. With a sample size of 70 individuals per arm, the study had a 90% power (1-β) to detect, with $\alpha = 0.05$, a difference of at least 25% in urinary 8-epi-$PGF_{2\alpha}$ between treated and untreated individuals. Two groups of 72 subjects treated or not treated with vitamin E were studied. Clinical and biochemical variables were reassessed yearly and on the occasion of urine collection.

Urinary 8-epi-$PGF_{2\alpha}$ was selectively measured as we have described previously [21] using immunoaffinity chromatography for selective extraction/purification and a stable isotope dilution assay with gas chromatography-negative ion chemical ionization mass spectrometry for quantitation, with $^2H_4$-8-*epi*-$PGF_{2\alpha}$ as the internal standard. Urinary excretion of 8-epi-$PGF_{2\alpha}$ was expressed as pg/mg creatinine. Creatinine was measured highly selectively by stable-isotope dilution HPLC-electrospray-tandem mass spectrometry, using $^2H_3$-creatinine as the internal stand-

Table 2: Baseline characteristics of the two study groups

	VITAMIN E (n = 72)	NO VITAMIN E (n = 72)
Age (y)	59 ± 6	61 ± 7
Sex (M/F)	32/40	31/41
Body mass index (kg/m^2)	27 ± 4	27 ± 4
Smokers (yes/no)	15/57	9/63
Systolic blood pressure (mm Hg)	146 ± 16	145 ± 15
Diastolic blood pressure (mm Hg)	88 ± 8	87 ± 7
Blood glucose (mg/dL)	91 ± 32	103 ± 35**
Total blood cholesterol (mg/dL)	243 ± 47	225 ± 45*
Aspirin treatment (yes/no)	36/36	34/38

Values are expressed as mean ± SD or number. *p < 0.05, **p < 0.01

ard. Urine was stored at -20°C until analyzed. Analyses were done on blind-coded samples.

Means were compared by the non-parametric Mann-Whitney U test to avoid assumptions about the distribution of the variables. Levels of 8-epi-$PGF_{2\alpha}$ were expressed as median (range) values. Multiple regression analysis was used to 1) evaluate the effect of vitamin E on urinary excretion of 8-epi-$PGF_{2\alpha}$, taking into account the following potential confounding variables (age, sex, aspirin treatment, smoking, systolic and diastolic blood pressure, blood glucose, blood cholesterol and obesity), and 2) assess whether any of these variables was independently associated with lipid peroxidation. Linear correlation analysis was also used. Probability values of $p \leq 0.05$ (two tails) were considered to be statistically significant.

Results

1. Antioxidant Effect of Vitamin E

In vivo lipid peroxidation was not reduced significantly by vitamin E (Figure 1), as indicated by the similar urinary excretion of 8-epi-$PGF_{2\alpha}$ in the supplemented group and in the controls [141 (67–498) vs 148 (76–561) pg/mg creatinine, p = 0.10]. These subjects had mean ± SD follow-up durations of 2.8 ± 1.0 and 2.7 ± 1.1 years, respectively. Baseline characteristics were well balanced across the two study groups, except for a slight difference in blood levels of glucose and cholesterol (Table 2). For this reason, we excluded with reasonable confidence a potential bias due to different baseline levels of urinary 8-epi-$PGF_{2\alpha}$. Multiple regression analysis, which takes into account possible confounding variables at the time of urine collection (age, sex, smoking, blood glucose, blood cholesterol, systolic and diastolic blood pressure, body mass index, aspirin treatment), confirmed that vitamin E had no significant overall effect on urinary 8-epi-$PGF_{2\alpha}$ (β = -0.14, p = 0.12, Table 3).

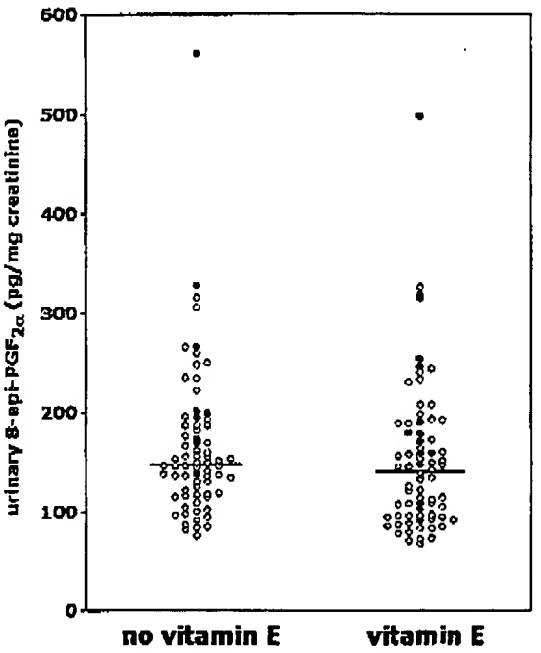

Figure 1
Urinary excretion of 8-epi-$PGF_{2\alpha}$ in PPP participants supplemented or not with vitamin E. Smokers are indicated by filled circles. The horizontal lines represent the median.

Smoking was the only strong determinant of lipid peroxidation in the overall sample (Table 3, discussed below). Since it is known that vitamin E does not reduce excessive lipid peroxidation in smokers [22,23], we investigated whether the presence of smokers in our sample might mask an antioxidant effect of vitamin E in the nonsmokers (n = 120; 63 untreated, 57 treated with vitamin E). The

Table 3: Multiple regression analysis: variables associated with urinary excretion of 8-epi-$PGF_{2\alpha}$

	all subjects (n = 144)		nonsmokers (n = 120)	
VARIABLE	β	p	β	p
Smoking	0.46	<0.0001	–	–
Vitamin E treatment	-0.14	0.12	-0.10	0.32
Systolic blood pressure (mmHg)	0.13	0.19	0.26	0.02
Obesity	0.12	0.21	0.15	0.15
Sex (M)	0.11	0.22	0.13	0.21
Aspirin treatment	-0.10	0.24	-0.07	0.45
Blood glucose (mg/dL)	0.09	0.35	0.17	0.11
Diastolic blood pressure (mmHg)	-0.05	0.63	-0.07	0.50
Age (y)	0.04	0.67	0.09	0.38
Blood cholesterol (mg/dL)	0.03	0.74	0.11	0.30

levels of 8-epi-$PGF_{2\alpha}$ in nonsmokers, however, were not significantly reduced by prolonged vitamin E supplementation [122 (67–326) *vs* 146 (76–316), p = 0.09)]. Multivariate analysis confirmed that vitamin E did not reduce lipid peroxidation in this sample (β = -0.10, p = 0.32, Table 3).

2. *Lipid Peroxidation in Subjects at CV Risk*

Since vitamin E has thus far proved effective as an *in vivo* antioxidant in humans when lipid peroxidation is excessive [17–19,24], but not when it is normal [20], we addressed the question whether in subjects eligible for primary prevention of CVD, lipid peroxidation was increased enough to decrease appreciably with vitamin E. As a whole, this sample of patients with at least one CVD risk factor did not have an abnormal rate of lipid peroxidation. The levels of urinary 8-epi-$PGF_{2\alpha}$ in untreated nonsmokers [146 (76–316) pg/mg creatinine] were similar to those of controls in other studies where this biomarker was selectively measured by mass spectrometry [20,22]. They were also similar to those we found in healthy nonsmoking volunteers [139 (71–256) pg/mg creatinine; mean ± SD age, 37 ± 11y; n = 20, unpublished data].

To better characterize the level of CV risk in our sample, we calculated a global CV risk score for each subject. We used Framingham's multiple-risk-factor assessment equation, a function assessing the risk of developing coronary heart disease on the basis of the presence and the level of major CV risk factors [25]. As shown in Figure 2, urinary 8-epi-$PGF_{2\alpha}$ did not correlate with risk level in untreated or treated subjects.

We therefore analyzed more closely factors possibly associated with lipid peroxidation in our sample, which is rather heterogeneous but is fairly representative of a population with major risk factors for cardiovascular diseases.

3. *Factors Associated with Lipid Peroxidation in Subjects at CV Risk*

Smoking

Multiple regression analysis of the whole group of 144 subjects showed cigarette smoking was the only strong determinant of excessive lipid peroxidation (β = 0.46; p < 0.0001, Table 3). Urinary excretion of 8-epi-$PGF_{2\alpha}$ was higher in smokers (n = 24; 14 ± 6 cigarettes daily) than nonsmokers (n = 120) [185 (91–561) *vs* 138 (67–326) pg/mg creatinine; p = 0.003]. Vitamin E did not significantly reduce levels of 8-epi-$PGF_{2\alpha}$ in smoking PPP participants [179 (91–498) *vs* 199 (138–561) pg/mg creatinine; p = 0.44; n = 15 and 9, respectively].

Other factors

The presence of smoking, a strong determinant of lipid peroxidation, very likely hampered the detection of other clinically important variables possibly associated with urinary 8-epi-$PGF_{2\alpha}$ in our initial sample. We therefore investigated the relationship between these variables and 8-epi-$PGF_{2\alpha}$ levels in the 120 nonsmokers.

Systolic blood pressure

Lipid peroxidation appeared to be related to systolic blood pressure in nonsmokers (β = 0.26; p = 0.02, Table 3). To confirm this in a less heterogeneous sample, we analyzed a subgroup of subjects who had hypertension as the only risk factor (n = 45). All except one were under antihypertensive treatment, with mean ± SD systolic and diastolic blood pressure 145 ± 16 (range 107–187) and 87 ± 8 (range 64–105) mm Hg, respectively. In this subgroup, the correlation between systolic blood pressure

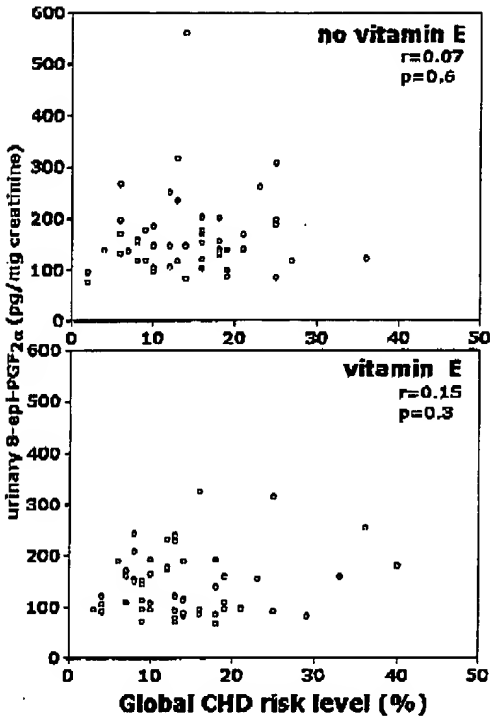

Figure 2
Correlation between global CV risk level and urinary excretion of 8-epi-$PGF_{2\alpha}$ in PPP participants without (upper panel) or with (lower panel) vitamin E supplementation. Risk levels, calculated according to Framingham's multiple-risk-factor assessment equation, represent coronary heart disease (CHD) individual risk over the next ten years [25].

and urinary excretion of 8-epi-$PGF_{2\alpha}$ was highly significant (r = 0.50, p = 0.0005. Figure 3), and subjects with systolic blood pressure ≥ 140 mm Hg had higher excretion of 8-epi-$PGF_{2\alpha}$ than those with <140 mm Hg [151 (140–187) *vs* 127 (107–139) pg/mg creatinine, n = 27 and 18, respectively, p = 0.004]. Vitamin E did not significantly affect 8-epi-$PGF_{2\alpha}$ excretion [109 (67–240) *vs* 138 (76–266) pg/mg creatinine in 21 treated and 24 untreated hypertensive subjects; p = 0.19].

Hyperglycemia

In nonsmoking participants, blood glucose measured at the time of urine collection (mean ± SD: 108 ± 24 mg/dL, n = 94) did not appear to be related to lipid peroxidation, based on multiple regression analysis (β = 0.17, p = 0.11. Table 3). However, subjects with blood glucose ≥ 140 mg/dL had higher excretion of 8-epi-$PGF_{2\alpha}$ than those with <140 mg/dL [180 (121–326) *vs* 140 (70–266), n = 10 and

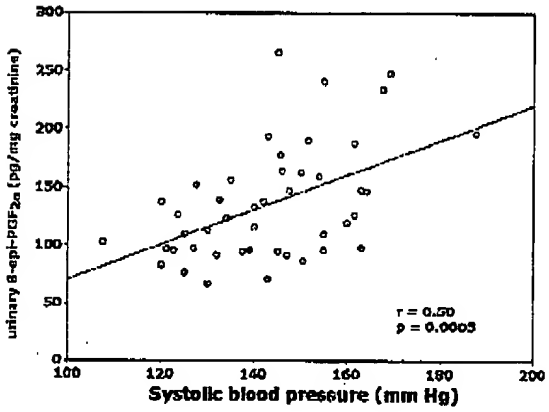

Figure 3
Correlation between systolic blood pressure and urinary excretion of 8-epi-$PGF_{2\alpha}$ in 45 PPP participants with hypertension as the only risk factor.

84, respectively, p = 0.009)], likely because only high blood glucose levels may be associated with excessive lipid peroxidation.

Other factors

In nonsmoking PPP participants, blood cholesterol at the time of urine collection (mean ± SD: 231 ± 40 mg/dL, range 144–351) was not significantly associated with lipid peroxidation (Table 3). Aspirin administration (n = 72) did not affect urinary excretion of 8-epi-$PGF_{2\alpha}$ (Table 3), as already reported [14,26]. We found no evidence (Table 3) of increased lipid peroxidation in relation to age, sex or obesity.

Discussion

Long-term supplementation with vitamin E-at a dose largely exceeding that of a vitamin E-rich diet (300 mg/day)-did not substantially reduce lipid peroxidation in people with one or more major cardiovascular risk factors. This may help explain why vitamin E was not effective for CVD prevention in the PPP study. As opposed to other studies showing an antioxidant effect of vitamin E in small, uncontrolled subgroups of patients, our observations were obtained in a sample of subjects that more realistically represent a population with cardiovascular risk factors. In fact, as usually occurs in clinical practice, most candidates for cardiovascular prevention are treated-although not necessarily controlled-for their modifiable risk factors. The lack of antioxidant effect in our sample may be explained by the rather surprising finding that lipid peroxidation was normal in a large proportion of these subjects.

We investigated whether oxidative stress was increased in high-risk subjects, given that this category could, in principle, be more sensitive to antioxidant therapy. However, we found no evidence of a correlation between CV risk score and urinary excretion of 8-epi-$PGF_{2\alpha}$ in untreated subjects, suggesting that lipid peroxidation was not associated with their global CV risk. Although, on average, lipid peroxidation was normal in our population, it was clearly increased in relation to extreme conditions reportedly associated with oxidative stress. Lipid peroxidation was, in fact, significantly higher in smokers, in agreement with consistent evidence of elevated oxidative stress in cigarette smokers, mostly obtained using 8-epi-$PGF_{2\alpha}$ and/or other F_2-isoprostanes as biomarkers [21,22,27]. We also confirmed previous observations that excessive lipid peroxidation in smokers cannot be reduced by vitamin E [22,23].

A secondary, but original finding of this study is the direct relationship between urinary excretion of an F_2-isoprostane and systolic blood pressure in treated hypertensive patients with different degrees of blood pressure control. Whether this relationship also exists in untreated hypertensive patients should be investigated. An association between oxidative stress and arterial hypertension has been suggested by several clinical and experimental studies [28–32]. The hypothesis that free radical-mediated mechanisms may play a role in the pathophysiology of hypertension has recently gained support from observations that antioxidants lower blood pressure in hypertensive patients [30,33–36]. However, vitamin E in particular does not seem effective [37], possibly because it was not antioxidant in these circumstances.

As to other factors reportedly related to *in vivo* lipid peroxidation, our findings agree with previous evidence. In particular, we confirmed that enhanced urinary excretion of 8-epi-$PGF_{2\alpha}$ is associated with impaired glycemic control in diabetic patients [17]. We did not find a correlation between urinary excretion of 8-epi-$PGF_{2\alpha}$ and blood cholesterol, which, on average, was slightly elevated in our sample. Such a correlation was, in fact, found in subjects with homozygote familial hypercholesterolemia and in subjects with very high blood cholesterol, but not in those with normal cholesterol levels [24].

Conclusions

Prolonged supplementation with 300 IU/day vitamin E did not reduce lipid peroxidation in subjects with one or more major cardiovascular risk factors. On average, however, lipid peroxidation was near-normal in this population. These data may help explain the overall lack of benefit of vitamin E in recent cardiovascular prevention trials [6–8]. They also suggest the need to reassess whether lipid peroxidation is indeed an epidemiologically relevant determinant of cardiovascular diseases and, consequently, to reconsider the utility of antioxidants as a general preventive measure.

Competing Interests

None declared.

Collaborative Group

Participating physicians: Marina Bosisio Pioltelli (general practitioner, Monza), Alberto Capra (Ospedale Civile, Voghera), Mario Cristofari (Ospedale di Desio), Gaetana Palumbo (Ospedale S. Carlo Borromeo, Milano) and Susanna Rossi (Ospedale di Rovereto)

Coordinating group: Chiara Chiabrando, Fausto Avanzini, Roberto Fanelli and Maria Carla Roncaglioni (Istituto Mario Negri, Milano)

Acknowledgements

Claudia Rivolta was supported by the Fondazione "Angela and Angelo Valenti."

References

1. Diaz MN, Frei B, Vita JA, Keaney JF Jr: **Antioxidants and atherosclerotic heart disease.** *N Engl J Med* 1997, 337:408-416
2. Jialal I, Fuller CJ, Huet BA: **The effect of alpha-tocopherol supplementation on LDL oxidation. A dose-response study.** *Arterioscler Thromb Vasc Biol* 1995, 15:190-198
3. Marchioli R: **Antioxidant vitamins and prevention of cardiovascular disease: laboratory, epidemiological and clinical trial data.** *Pharmacol Res* 1999, 40:227-238
4. Pratico D, Tangirala RK, Rader DJ, Rokach J, FitzGerald GA: **Vitamin E suppresses isoprostane generation in vivo and reduces atherosclerosis in ApoE-deficient mice.** *Nat Med* 1998, 4:1189-1192
5. Steinberg D: **Is there a potential therapeutic role for vitamin E or other antioxidants in atherosclerosis?** *Curr Opin Lipidol* 2000, 11:603-607
6. Gruppo Italiano per lo Studio della Sopravvivenza nell'Infarto miocardico: **Dietary supplementation with n-3 polyunsaturated fatty acids and vitamin E after myocardial infarction: results of the GISSI-Prevenzione trial.** *Lancet* 1999, 354:447-455
7. Yusuf S, Dagenais G, Pogue J, Bosch J, Sleight P: **Vitamin E supplementation and cardiovascular events in high-risk patients. The Heart Outcomes Prevention Evaluation Study Investigators.** *N Engl J Med* 2000, 342:154-160
8. Collaborative Group of the Primary Prevention Project: **Low-dose aspirin and vitamin E in people at cardiovascular risk: a randomised trial in general practice.** *Lancet* 2001, 357:89-95
9. Witztum JL: **To E or not to E-how do we tell?** *Circulation* 1998, 98:2785-2787
10. Halliwell B: **The antioxidant paradox.** *Lancet* 2000, 355:1179-1180
11. Hooper L, Ness AR, Smith GD: **Antioxidant strategy for cardiovascular diseases.** *Lancet* 2001, 357:1705-1706
12. Roncaglioni MC, Tombesi M, Chiabrando C, Bertele' V, Tognoni G: **Antioxidant strategy for cardiovascular diseases.** *Lancet* 2001, 357:1706
13. Meagher EA, FitzGerald GA: **Indices of lipid peroxidation in vivo: strengths and limitations.** *Free Radic Biol Med* 2000, 28:1745-1750
14. Delanty N, Reilly M, Pratico D, FitzGerald DJ, Lawson JA, FitzGerald GA: **8-Epi $PGF_{2\alpha}$: specific analysis of an isoeicosanoid as an index of oxidant stress in vivo.** *Br J Clin Pharmacol* 1996, 42:15-19
15. Patrono C, FitzGerald GA: **Isoprostanes: potential markers of oxidant stress in atherothrombotic disease.** *Arterioscler Thromb Vasc Biol* 1997, 17:2309-2315
16. Pratico D, Lawson JA, Rokach J, FitzGerald GA: **The isoprostanes in biology and medicine.** *Trends Endocrinol Metab* 2001, 12:243-247

17. Davi G, Ciabattoni G, Consoli A, Mezzetti A, Falco A, Santarone S, Pennese E, Vitacolonna E, Bucciarelli T, Costantini F, Capani F, Patrono C: **In vivo formation of 8-iso-prostaglandin $F_{2\alpha}$ and platelet activation in diabetes mellitus: effects of improved metabolic control and vitamin E supplementation.** *Circulation* 1999, **99**:224-229
18. Davi G, Alessandrini P, Mezzetti A, Minotti G, Bucciarelli T, Costantini F, Cipollone F, Bon GB, Ciabattoni G, Patrono C: **In vivo formation of 8-Epi-prostaglandin $F_{2\alpha}$ is increased in hypercholesterolemia.** *Arterioscler Thromb Vasc Biol* 1997, **17**:3230-3235
19. Ciabattoni G, Davi G, Collura M, Iapichino L, Pardo F, Ganci A, Romagnoli R, Maclouf J, Patrono C: **In vivo lipid peroxidation and platelet activation in cystic fibrosis.** *Am J Respir Crit Care Med* 2000, **162**:1195-1201
20. Meagher EA, Barry OP, Lawson JA, Rokach J, FitzGerald GA: **Effects of vitamin E on lipid peroxidation in healthy persons.** *JAMA* 2001, **285**:1178-1182
21. Bachi A, Zuccato E, Baraldi M, Fanelli R, Chiabrando C: **Measurement of urinary 8-Epiprostaglandin $F_{2\alpha}$, a novel index of lipid peroxidation in vivo, by immunoaffinity extraction/gas chromatography-mass spectrometry. Basal levels in smokers and nonsmokers.** *Free Radic Biol Med* 1996, **20**:619-624
22. Reilly M, Delanty N, Lawson JA, FitzGerald GA: **Modulation of oxidant stress in vivo in chronic cigarette smokers.** *Circulation* 1996, **94**:19-25
23. Patrignani P, Panara MR, Tacconelli S, Seta F, Bucciarelli T, Ciabattoni G, Alessandrini P, Mezzetti A, Sandini G, Sciulli MG, Cipollone F, Davi G, Gallina P, Bon GB, Patrono C: **Effects of vitamin E supplementation on F_2-isoprostane and thromboxane biosynthesis in healthy cigarette smokers.** *Circulation* 2000, **102**:539-545
24. Reilly MP, Pratico D, Delanty N, DiMinno G, Tremoli E, Rader D, Kapoor S, Rokach J, Lawson J, FitzGerald GA: **Increased formation of distinct F_2 isoprostanes in hypercholesterolemia.** *Circulation* 1998, **98**:2822-2828
25. Anderson KM, Wilson PW, Odell PM, Kannel WB: **An updated coronary risk profile. A statement for health professionals.** *Circulation* 1991, **83**:356-362
26. Wang Z, Ciabattoni G, Creminon C, Lawson J, Fitzgerald GA, Patrono C, Maclouf J: **Immunological characterization of urinary 8-epi-prostaglandin $F_{2\alpha}$ excretion in man.** *J Pharmacol Exp Ther* 1995, **275**:94-100
27. Morrow JD, Frei B, Longmire AW, Gaziano JM, Lynch SM, Shyr Y, Strauss WE, Oates JA, Roberts LJ 2nd: **Increase in circulating products of lipid peroxidation (F_2-isoprostanes) in smokers. Smoking as a cause of oxidative damage.** *N Engl J Med* 1995, **332**:1198-1203
28. Russo C, Olivieri O, Girelli D, Faccini G, Zenari ML, Lombardi S, Corrocher R: **Anti-oxidant status and lipid peroxidation in patients with essential hypertension.** *J Hypertens* 1998, **16**:1267-1271
29. Romero JC, Reckelhoff JF: **State-of-the-Art lecture. Role of angiotensin and oxidative stress in essential hypertension.** *Hypertension* 1999, **34**:943-949
30. Schnackenberg CG, Wilcox CS: **Two-week administration of tempol attenuates both hypertension and renal excretion of 8-iso prostaglandin $F_{2\alpha}$.** *Hypertension* 1999, **33**:424-428
31. Touyz RM: **Oxidative stress and vascular damage in hypertension.** *Curr Hypertens Rep* 2000, **2**:98-105
32. Berry C, Brosnan MJ, Fennell J, Hamilton CA, Dominiczak AF: **Oxidative stress and vascular damage in hypertension.** *Curr Opin Nephrol Hypertens* 2001, **10**:247-255
33. Galley HF, Thornton J, Howdle PD, Walker BE, Webster NR: **Combination oral antioxidant supplementation reduces blood pressure.** *Clin Sci (Colch)* 1997, **92**:361-365
34. Kitiyakara C, Wilcox CS: **Antioxidants for hypertension.** *Curr Opin Nephrol Hypertens* 1998, **7**:531-538
35. Duffy SJ, Gokce N, Holbrook M, Huang A, Frei B, Keaney JF Jr, Vita JA: **Treatment of hypertension with ascorbic acid.** *Lancet* 1999, **354**:2048-2049
36. Ortiz MC, Manriquez MC, Romero JC, Juncos LA: **Antioxidants block angiotensin II-induced increases in blood pressure and endothelin.** *Hypertension* 2001, **38**:655-659
37. Palumbo G, Avanzini F, Alli C, Roncaglioni MC, Ronchi E, Cristofari M, Capra A, Rossi S, Nosotti L, Costantini C, Cavalera C: **Effects of vitamin E on clinic and ambulatory blood pressure in treated hypertensive patients. Collaborative Group of the Primary Prevention Project (PPP) – Hypertension study.** *Am J Hypertens* 2000, **13**:564-567

Can Garlic Reduce Levels of Serum Lipids? A Controlled Clinical Study

ADESH K. JAIN, M.D., RAMON VARGAS, M.D., SANDRA GOTZKOWSKY, R.N., F. GILBERT MCMAHON, M.D., F.A.C.P., *New Orleans, Louisiana*

PURPOSE: To assess the effects of standardized garlic powder tablets on serum lipids and lipoproteins, glucose, and blood pressure.

SUBJECTS AND METHODS: Forty-two healthy adults (19 men, 23 women), mean age of 52 ± 12 years, with a serum total cholesterol (TC) level of greater than or equal to 220 mg/dL received, in a randomized, double-blind fashion, either 300 mg three times a day of standardized garlic powder in tablet form or placebo. Diets and physical activity were unchanged. This study was conducted in an outpatient, clinical research unit.

RESULTS: The baseline serum TC level of 262 ± 34 mg/dL was reduced to 247 ± 40 mg/dL ($p < 0.01$) after 12 weeks of standard garlic treatment. Corresponding values for placebo were 276 ± 34 mg/dL before and 274 ± 29 mg/dL after placebo treatment. Low-density lipoprotein cholesterol (LDL-C) was reduced by 11% by garlic treatment and 3% by placebo ($p < 0.05$). There were no significant changes in high-density lipoprotein cholesterol, triglycerides, serum glucose, blood pressure, and other monitored parameters.

CONCLUSIONS: Treatment with standardized garlic 900 mg/d produced a significantly greater reduction in serum TC and LDL-C than placebo. The garlic formulation was well tolerated without any odor problems.

From the Clinical Research Center and Tulane University School of Medicine, New Orleans, Louisiana.

This study was supported by a grant from Lichtwer Pharma GbmH, Berlin, Germany.

Requests for reprints should be addressed to Adesh K. Jain, M.D., Clinical Research Center, 147 South Liberty Street, New Orleans, Louisiana 70112.

Manuscript submitted June 4, 1992, and accepted in revised form November 19, 1992.

Garlic (*Allium sativum*) [1–3] has been used in herbal medicine for centuries for various ailments. In recent years, garlic has been the focus of serious medical and clinical attention because of reports of beneficial effects on several cardiovascular risk factors [4,5]. Garlic extracts have been reported to reduce levels of serum lipids [3–13], blood pressure [4,5,7–13,16,18], and plasma viscosity [19], inhibit platelet aggregation [9,16,18,20,21], increase fibrinolytic activity [9,21], and produce vasodilation [21–23]. Thus, garlic is assumed to have antiatherosclerotic properties, although its full potential in the prevention or treatment of circulatory or vascular diseases is yet to be defined.

Garlic's principal active agent appears to be allicin, a sulfur-containing compound that with its breakdown products, gives garlic its characteristic odor [1]. Allicin is formed enzymatically from an odorless precursor, alliin, when garlic cloves are mechanically disrupted. Since the alliin content of natural garlic may vary 10-fold and the quantity of allicin released can be influenced by specific extraction methods [24,25], standardizing garlic products by using their potential for releasing allicin has been suggested to ensure the accuracy of dosage and effectiveness in long-term therapy [26].

A dried garlic powder tablet standardized to provide 1.3% alliin, which corresponds to an allicin release of 0.6%, has been available in Germany as a dietary supplement over the counter (OTC) to improve cardiovascular risk factors [27]. More recently, these garlic tablets have been available OTC in the United States, but no health claims are made.

We conducted a 12-week, double-blind study comparing the effects of standardized garlic tablets (Kwai, Lichtwer Pharma GmbH, Berlin, Germany), 900 mg/d, with that of placebo on serum lipids, serum glucose, blood pressure, and other parameters in 42 subjects with hypercholesterolemia.

SUBJECTS AND METHODS

Subjects with known serum total cholesterol (TC) levels greater than or equal to 220 mg/dL were screened by a history and physical examination, Chem-23 (serum glucose, blood urea nitrogen, creatinine, sodium, potassium, chloride, carbon dioxide, calcium, phosphorus, TC, triglycerides [TG], total bilirubin, direct bilirubin, indirect bilirubin, alkaline phosphatase, serum glutamic oxaloacetic transaminase [SGOT], serum glutamic pyruvic

transaminase [SGPT], γ-glutamyl transferase, lactic dehydrogenase, total protein, albumin, globulin, albumin/globulin ratio, uric acid, and iron), complete blood count (CBC), urinalysis, and electrocardiogram (EKG) prior to the study enrollment. Patients were excluded if they were older than 70 years or had a history of drug or alcohol abuse, impaired hepatic function test results (SGOT/SGPT) greater than 20% above normal, unstable angina, myocardial infarction, or coronary bypass surgery within 6 months, diabetes mellitus, known secondary hypercholesterolemia due to nephrotic syndrome or hypothyroidism, serum creatinine level greater than 2.0 mg/dL, or use of lipid-lowering agents within 1 month prior to enrollment. All subjects provided written informed consent before enrollment and were advised not to change their dietary habits or physical activity during the course of the study.

Qualifying subjects were then instructed to return to the clinic in a fasting state in 14 ± 2 days for a lipid profile (TC, TG, high-density lipoprotein cholesterol [HDL-C], low-density lipoprotein cholesterol [LDL-C]) and serum glucose measurement. Those with serum TC values of greater than or equal to 220 mg/dL at two consecutive visits were randomized to either placebo or matching garlic tablets, 300 mg three times a day, for 12 weeks, after all baseline parameters were obtained. During the double-blind phase, clinic visits were 6 weeks apart. Lipid profile and serum glucose determinations were repeated at Weeks 6 and 12. At the end of the double-blind phase, each subject had an exit physical examination, safety laboratory tests (Chem-23, CBC, urinalysis), and EKG. Two sitting blood pressure measurements and a pulse rate were obtained after 10 minutes of rest in the clinic using an appropriate-size cuff, a conventional mercury sphygmomanometer, and standard recommended technique [28]. Body weight, side effects, and compliance were also monitored at each clinic visit, and patients were questioned as to any changes in diet or physical activity.

All laboratory analyses were done by a CDC-(Centers for Disease Control) and NWLRC-(Northwestern Lipid Research Clinic, Seattle) standardized laboratory, SmithKline Beecham Labs. The serum TC [29] and TG [30] were measured by enzymatic procedures. HDL-C was isolated initially by precipitating LDL-C and very-low-density-lipoprotein cholesterol (VLDL-C) with phosphotungstate/magnesium chloride. An aliquot of the supernatant was then assayed for cholesterol content [31]. LDL-C was calculated by the Friedewald equation, LDL-C = TC − HDL-C + TG/5. Patients with TG greater than 400 mg/dL were excluded.

Statistical Analysis

The average of two visits prior to randomization represented the baseline value for the serum lipid, glucose, blood pressure, body weight, and other parameters. The differences from baseline value at Weeks 6 and 12 were then calculated and these differences were analyzed for treatment effects by an analysis of variance, followed by Mann-Whitney and Wilcoxon U-tests [32].

TABLE I
Demographic Characteristics

	Standardized Garlic Tablets (900 mg/d)	Placebo
No.	20 (14 W, 6 B)	22 (15 W, 7 B)
Mean age (y)	48 ± 15*	55 ± 9
Sex	11 M, 9 F	8 M, 14 F
Body weight (kg)	78 ± 17	77 ± 14
Blood pressure (mm Hg)	128/81 ± 11/7	126/82 ± 9/7
Height (cm)	168 ± 9	165 ± 13
Nonsmokers	11	20

W = white; B = black; M = male; F = female.
*Mean ± SD of mean.

RESULTS

The demographic characteristics of the study population are shown in Table I. The two treatment groups were fairly comparable for the listed variables. Mean age was slightly lower in the garlic-treated group and there were fewer smokers in the placebo group.

The effects of garlic tablets and placebo on levels of serum lipids, blood pressure, and body weight at baseline and at the end of 6 and 12 weeks of treatment are shown in Table II. Changes from baseline for serum TC and LDL-C at the end of 12 weeks are shown in Figure 1. No significant treatment differences in the measured parameters were seen at Week 6 between the placebo and garlic groups. At Week 12, however, serum TC was lowered by 6% with garlic tablets and 1% with placebo ($p < 0.01$). This reduction in serum TC was caused mainly by a reduction in LDL-C, which was decreased by 11% in the garlic-treated group and 3% in the placebo group ($p < 0.05$). There was a small, but nonsignificant, increase in TG in both groups. HDL-C, serum glucose, blood pressure, and body weight did not change significantly.

Only 1 of 20 patients treated with garlic tablets complained of increased belching with garlic taste. Two patients receiving placebo had mild abdominal discomfort. One patient taking placebo reported prolonged oozing from a razor cut during shaving, and another patient had a minor rash. In general, garlic tablets were quite well tolerated without any significant odor problems.

COMMENTS

The hypolipemic efficacy of garlic tablets, standardized to release 0.6% allicin, has been well studied in

TABLE II

Efficacy Variables*

	Standardized Garlic (900 mg/d) (n = 20)			Placebo (n = 22)		
	Before	6 Wk	12 Wk	Before	6 Wk	12 Wk
TC (mg/dL)	262 ± 35	248 ± 31	247 ± 40†	276 ± 34	262 ± 38	274 ± 29
LDL-C (mg/dL)	188 ± 37	172 ± 33	168 ± 43‡	191 ± 34	180 ± 39	185 ± 25
HDL-C (mg/dL)	47 ± 12	45 ± 13	46 ± 13	49 ± 14	48 ± 15	50 ± 17
TG (mg/dL)	151 ± 81	166 ± 137	165 ± 86	195 ± 112	176 ± 107	199 ± 101
Glucose (mg/dL)	100 ± 11	102 ± 13	98 ± 12	98 ± 9	97 ± 7	97 ± 7
Blood pressure (mm Hg)	129 ± 13	129 ± 15	130 ± 17	128 ± 10	127 ± 12	127 ± 12
	82 ± 6	83 ± 8	81 ± 10	83 ± 8	81 ± 7	82 ± 6
Body weight (kg)	79 ± 17	79 ± 17	79 ± 17	77 ± 14	77 ± 14	77 ± 15
Heart rate (beats/min)	71 ± 8	66 ± 6	69 ± 6	72 ± 7	68 ± 6	70 ± 8

*Mean ± SD.
†$p < 0.01$ kwai versus placebo for differences from baseline treatment effects.
‡$p < 0.05$ kwai versus placebo for differences from baseline treatment effects.

TABLE III

Reported Efficacy of Garlic Treatment

References	Study Design	Sample Size	Dose of Garlic* (mg/d)	Treatment Duration (wk)	% Reduction TC	% Reduction TG	Drop in BP (mm Hg)
[7]	DB, standard	40	600	12	−6	−8	6/15
[8]	DB, placebo	40	600	12	−10	−8	9/16
[9]	Open	20	600	4	−11	+3	8/5
[10]	DB, placebo	261	800	16	−11.6	−16.8	N/A
[12]	DB, R, P	47	600	12	−12	−21	12/11
[13]	DB, placebo	40	900	16	−21	−24	4/3
[14]	Open	40	600	12	−6.4	−16	N/A

BP = blood pressure, DB = double-blind; R = randomized; P = placebo; N/A = not available.
*Given as kwai.

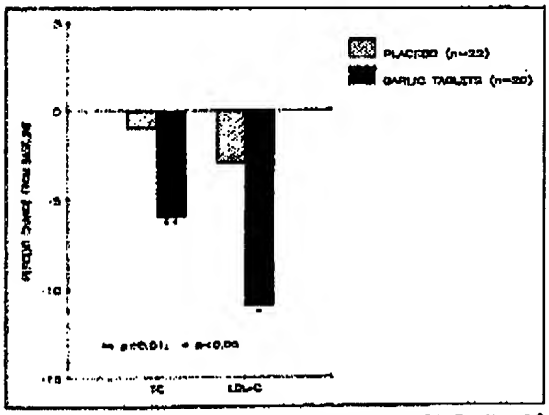

Figure 1. Percent reduction in serum TC and LDL-C after 12 weeks of treatment with standardized garlic powder tablets or placebo.

Germany, where it is available OTC as a dietary supplement to improve cardiovascular risk factors [27].

In this double-blind randomized study, 12 weeks of treatment with garlic 900 mg/d produced a modest but significantly greater reduction in serum TC and LDL-C than that with placebo. This reduction in serum cholesterol, although somewhat lower, is congruous with the data reported by others (Table III).

In a double-blind, multicenter study in 261 German patients with hyperlipidemia, Mader *et al* [10] reported a 12% reduction in TC and 17% decrease in TG after 16 weeks of treatment with standardized garlic powder 800 mg/d. The subgroup with initial cholesterol levels in the range of 250 to 300 mg/dL showed the greatest response, with a 14% reduction in TC. The onset of the hypolipidemic effect was evident as early as 4 weeks and became progressive and greater with time. There was no special dietary monitoring. Vorberg and Schneider [13] reported even greater reductions, e.g., 21% in TC and 24% in TG following 4 months of treatment with standardized garlic powder 900 mg/d. A low TC response in our study may be partly related to the shorter duration of treatment as well as to the greater body mass indices of our population when compared with the German data [14]. Administration of 600 mg/d of standardized garlic for 12 weeks has resulted in reductions in serum cholesterol that have ranged from 6.9% to 12%, and in TG from 8% to 12% [7–9,12,14].

In our study, there was a slight increase in TG in both the placebo and garlic-treated groups. However, neither the changes from baseline nor the differences between treatment groups were significant. The reason for this apparent discrepancy in our TG data as compared with that of others is not clear. Voluntary modifications of diet may have partly accounted for the changes in TG reported by others [7–10,12–14].

The mechanisms or active ingredients by which garlic lowers serum cholesterol levels are not known. Inhibition of hydroxymethylglutaryl coenzyme A reductase [33] and cholesterol biosynthesis [34] has been suggested.

Most studies with standardized garlic powder also reported some reduction in blood pressure in hypertensive subjects (Table III). Most subjects in our study were normotensive and only three patients in each group were receiving antihypertensive therapy. These patients also had normal blood pressure during treatment. Data analysis with or without inclusion of these three subjects in each group showed no significant change from average baseline blood pressure. There were no significant changes in serum glucose levels or body weight. Various safety parameters and EKG also showed no significant changes.

Garlic powder, given in the form of tablets in our study, was well tolerated and only one subject reported increased belching and a garlic odor. Mader *et al* [10] reported a 21% incidence of garlic smell following 800 mg/d of these garlic tablets. However, in their study, 9% of patients receiving placebo also reported the same garlic odor. In fact, garlic odor has been one of the major concerns of treatment with garlic extracts. The general incidence in the German reports appears to be about 10% to 15%. The low incidence in our study may be partly due to the high "expectation" of garlic smell or taste from ingesting garlic pills, and hence not "viewed" by the subjects as an adverse effect.

Since 900 mg/d was very well tolerated in our study, higher dosages of standardized garlic powder are worth exploring. Also, controlled clinical trials of longer duration are needed to assess the long-term benefit of garlic on vascular and circulatory disease processes.

In conclusion, treatment with garlic tablets standardized to deliver 0.6% allicin, the assumed active ingredient of garlic, produced a significantly greater reduction in TC and LDL-C than that with placebo. Treatment with garlic 900 mg/d for 12 weeks was very well tolerated with no significant garlic odor problem. Further studies are certainly warranted.

REFERENCES

1. Block E. The chemistry of garlic and onions. Sci Am 1985; 25: 94–9.
2. McElroy JC, Li Wan Po A. Dietary supplements (5). Garlic. Pharm J 1991; 16: 324–6.
3. Lau BHS, Adetumbi MA, Sanchez A. Allium sativum and atherosclerosis: a review. Nutr Res 1983; 3: 119–28.
4. 0. International Garlic Symposium: Pharmacy, pharmacology, and clinical application of allium sativum. Cardiology in Practice, Supplement, June 1991: 2–19.
5. Greenwood TW, editor. Garlic therapy. Br J Clin Prac 1990; 44 Suppl 69: 3–39.
6. Ernst E, Weihmayr TH, Matrai A. Garlic and blood lipids [letter]. BMJ 1985; 291: 139.
7. Kandziora J. Antihypertensive effectiveness and tolerance of a garlic medication. Ärztliche Forschung 1988; 1: 1–8.
8. Kandziora J. The blood pressure lowering and lipid lowering effect of a garlic preparation in combination with a diuretic. Ärztliche Forschung 1988; 3: 1–8.
9. Harenberg J, Giese C, Zimmermann R. Effect of dried garlic on blood coagulation, fibrinolysis, platelet aggregation and serum cholesterol levels in patients with hyperlipoproteinemia. Atherosclerosis 1988; 74: 247–9.
10. Mader FH. Treatment of hyperlipidaemia with garlic-powder tablets. Evidence from the German Association of General Practitioners' multicentric placebo-controlled double-blind study. Arzneimittelforschung 1990; 40: 1111–6.
11. Grunwald J. Garlic and cardiovascular risk factors [letter]. Br J Clin Pharmacol 1990; 29: 582–3.
12. Auer W, Eiber A, Hertkorn E, *et al*. Hypertension and hyperlipidaemia: garlic helps in mild cases. Br J Clin Pract 1990; 44 Suppl 69: 3–6.
13. Vorberg G, Schneider B. Therapy with garlic: results of a placebo-controlled, double-blind study. Br J Clin Pract 1990; 44 Suppl 69: 7–11.
14. Brosche T, Platt D, Dorner H. The effect of a garlic preparation on the composition of plasma lipoproteins and erythrocyte membranes in geriatric subjects. Br J Clin Pract 1990; 44 Suppl 69: 12–9.
15. Zimmermann W, Zimmermann B. Reduction in elevated blood lipids in hospitalized patients by a standardized garlic preparation. Br J Clin Pract 1990; 44 Suppl 69: 20–3.
16. Barrie SA, Wright JV, Pizzorno JE. Effects of garlic oil on platelet aggregation, serum lipids, and blood pressure in humans. J Orthomol Med 1987; 2: 15–21.
17. Bordia A. Effect of garlic on blood lipids in patients with coronary heart disease. Am J Nutr 1981; 34: 2100–3.
18. Mansell P, Reckless JPD. Garlic: effects on serum lipids, blood pressure, coagulation, platelet aggregation, and vasodilation. BMJ 1991; 303: 379–80.
19. Koscielny J, Jung EM, Jung F, *et al*. The effects of diverse garlic preparations on the viscosity of the blood. Medizinische Welt 1991; 42 Suppl 7A: 29–31.
20. Makheja AN, Bailey JM. Antiplatelet constituents of garlic and onions. Agents Actions 1990; 29; 360–3.
21. Kiesewetter H, Jung F, Pindur G, Jung EM, Mrowietz C, Wenzel E. Effect of garlic on thrombocyte aggregation, microcirculation, and other risk factors. Int J Clin Pharmacol Ther Toxicol 1991; 29: 151–5.
22. Jung F, Jung EM, Mrowietz C, Kiesewetter H, Wenzel E. Influence of garlic powder on cutaneous microcirculation: a randomised, placebo-controlled, double-blind, crossover study in apparently healthy subjects. Br J Clin Pract 1990; 44 Suppl 69: 30–5.
23. Wolf S, Reim M. Effect of garlic on conjunctival vessels: a randomized, placebo-controlled, double-blind trial. Br J Clin Pract 1990; 44 Suppl 69: 36–9.
24. Kleijnen J, Knipschild P, ter Riet G. Garlic, onions and cardiovascular risk factors. A review of the evidence from human experiments with emphasis on commercially available preparations. Br J Clin Pharmacol 1989; 28: 535–44.
25. Aye RD. Garlic preparations and processing. Cardiology in Practice 1989; 10 (Symposium Suppl): 7–8.
26. Muller B. Analytical methods for standardization of fresh garlic and garlic powder preparations. Deutsche Apotheker Zeitung 1991; 131 Suppl 2A: 8–10.
27. Fulder S. Garlic and the prevention of cardiovascular disease. Cardiology in Practice 1989; 7: 30–5.
28. 1988 Joint National Committee. The 1988 report of the Joint National Committee on Detection, Evaluation, and Treatment of High Blood Pressure. Arch Intern Med 1988; 148: 1023–8.
29. Naito HK. Cholesterol. In: Kaplan LA, Pesce AJ, editors. Clinical chemistry theory, analysis and correlation. 2nd ed. St. Louis: CV Mosby, 1989: 974–83.
30. Naito HK. Triglycerides. In: Kaplan LA, Pesce AJ, editors. Clinical chemistry theory, analysis and correlation. 2nd ed. St. Louis: CV Mosby, 1989: 997–1004.
31. Lopes-Virella MF, Stone P, Ellis S, Colwell JA. Cholesterol determination in high-density lipoproteins separated by three different methods. Clin Chem 1977; 23: 882–4.
32. Bolton S. Pharmaceutical statistics: practical and clinical applications. New York: Marcel Dekker, 1984; 394–6.
33. Brosche T, Siegers CP, Platt D. The effects of garlic therapy on cholesterol biosynthesis and on plasma and membrane lipids. Medizinische Welt 1991; 42 Suppl 7A: 10–1.
34. Gebhardt R. Inhibition of cholesterol biosynthesis by a water soluble garlic extract in primary cultures of rat hepatocytes. Arznei Forsch/Drug Res 1991; 41: 800–4.